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Genetic variation in *Brachyspira*: chromosomal rearrangements and sequence drift distinguish *B. pilosicoli* from *B. hyodysenteriae*

Richard L. Zuerner[a,*], Thaddeus B. Stanton[b], F. Chris Minion[c], Chunhao Li[d], Nyles W. Charon[d], Darren J. Trott[b,e], David J. Hampson[f]

[a] *Bacterial Diseases of Livestock Research Unit, National Animal Disease Center, US Department of Agriculture, Agricultural Research Service, P.O. Box 70, Ames, IA 50010, USA*

[b] *Pre-harvest Food Safety Research Unit, National Animal Disease Center, US Department of Agriculture, Agricultural Research Service, P.O. Box 70, Ames, IA 50010, USA*

[c] *Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA, 50010, USA*

[d] *Department of Microbiology, Immunology, and Cell Biology, West Virginia University, Morgantown, WV 26506-9177, USA*

[e] *School of Veterinary Science, The University of Queensland, St Lucia, Australia*

[f] *School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, Australia*

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Abstract

Brachyspira pilosicoli and *B. hyodysenteriae* are anaerobic pathogenic intestinal spirochetes differing in host range and disease manifestations. Little is known about the size, organization, or genetic content of the *B. pilosicoli* genome and only limited information is available regarding the genetic organization in *B. hyodysenteriae*. Both *B. hyodysenteriae* and *B. pilosicoli* exist as recombinant populations, and this may be due, in part, to an unusual phage-like gene transfer agent, VSH-1. To compare genetic organization in these two species, the number of mapped loci on an existing physical and genetic map of *B. hyodysenteriae* B78^T was expanded, and a combined physical and genetic map of *B. pilosicoli* P43/6/78^T was constructed. The *B. pilosicoli* genome size was about 2.5 Mb, nearly 750 kb smaller than the *B. hyodysenteriae* genome. Several chromosomal rearrangements have contributed to differences in the size, organization, and content of the two bacterial genomes, and such differences may influence the ability of these species to infect different hosts and cause disease. To evaluate these differences further, comparisons were focused on genes thought to contribute to host–parasite interactions. Four genetic loci (*bit*, *fruBC*, *vspA*, and *vspH*) were found in *B. hyodysenteriae*, but not in *B. pilosicoli*, while two genetic loci (*clpX* and *mglB*) were found in *B. pilosicoli*, but not in *B. hyodysenteriae*. Contrary to a previous study, an intact copy of the *hlyA* gene, encoding the *B. hyodysenteriae* β-hemolysin gene was detected in *B. pilosicoli.* Although the *hlyA* genes of these two species were nearly identical, sequence variation was detected in the intergenic region upstream of *hlyA* that may alter transcription and translation efficiency of this gene in *B. pilosicoli.* In addition, divergence in genes flanking *hlyA* may affect the chemical composition of lipid attached to the mature *B. pilosicoli* HlyA protein resulting in reduced hemolytic activity.

Keywords: Genome mapping; *Brachyspira*; Spirochete; Swine dysentery; Intestinal spirochetosis

1. Introduction

Members of the genus *Brachyspira* (formerly *Serpulina*) are anaerobic bacteria that inhabit the large intestines of a wide variety of animal species. Two members of this genus, *B. hyodysenteriae* and *B. pilosicoli*, are pathogenic and cause infections. *B. pilosicoli* was recently identified as the etiological agent of intestinal spirochetosis, and it has been isolated from a variety of hosts including humans, dogs, pigs, and various bird species [1,2]. Intestinal spirochetosis in humans occurs with high infection rates in developing countries [1], while in livestock it can contribute to significant losses for producers [3]. In contrast, *B. hyodysenteriae* is recognized predominantly as a pathogen of pigs, causing swine dysentery; a disease leading to

*Corresponding author. Tel.: +1-515-663-7392; fax: +1-515-663-7458.

E-mail address: rzuerner@nadc.ars.usda.gov (R.L. Zuerner).

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development of bloody diarrhea and death from weaning through growing and finishing stages of livestock production [4]. Although the incidence of these diseases was reduced using antibiotics in animal feeds, they may reappear as antibiotic use in livestock becomes restricted [5].

Studies using multilocus enzyme electrophoresis have shown that *B. hyodysenteriae* and *B. pilosicoli* are recombinant species [6,7]. Recombinant species are a result of extensive exchange of genetic alleles between members of the same species. This extensive exchange of genetic material contributes to a high degree of genetic variation amongst isolates and abrogates the development of clonal populations [6–8]. Some genetic loci show evidence of more frequent genetic exchange than other loci [6,7]. A likely candidate for promiscuous sharing of genetic material is the unusual gene transfer agent VSH-1, a phage-like particle, whose genome is integrated into the host chromosome [9]. Induction of phage synthesis results in random packaging of 7.5 kb genomic DNA fragments that are released in phage-like particles upon lysis of the host cell [9]. There is no evidence of phage replication, and thus replication of the phage is entirely dependent upon chromosomal replication. Although genes can be transduced between *B. hyodysenteriae* strains via VSH-1 [9], it is unclear whether VSH-1-mediated transduction is possible between *Brachyspira* species. Differences in restriction-modification systems may present a barrier to genetic exchange among different members of this genus.

Little information is available on genetic organization of *Brachyspira*. We previously constructed a physical and genetic map of the *B. hyodysenteriae* genome [10]. This map has been useful in localizing newly identified genetic loci, including the β-hemolysin gene *hlyA* and VSH-1 [11,12]. In the present study, several additional genes were localized on the *B. hyodysenteriae* map, and a combined physical and genetic map of the *B. pilosicoli* genome was constructed to provide a means to compare the genetic organization of these two bacteria. Detailed analysis of *hlyA* loci from both species was then undertaken to determine differences that potentially could explain different hemolysin phenotypes exhibited by these two *Brachyspira* species.

2. Materials and methods

2.1. Bacteriological culture and DNA preparation

Brachyspira hyodysenteriae and *B. pilosicoli* were grown in Brain Heart Infusion broth (Difco, Detroit, MI, USA) supplemented with 10% heat-inactivated bovine serum under a gaseous environment of 99% N_2: 1% O_2. Bacterial strains *B. pilosicoli* type strain P43/6/78^T and *B. hyodysenteriae* type strain B78^T were used in the construction of the physical maps. *B. hyodysenteriae* strain B204 and *B. pilosicoli* strain P43/6/78^T provided genomic DNA used as template for sequencing *hlyA* loci. Bacterial cultures in exponential growth were harvested by centrifugation, embedded in agarose beads, and genomic DNA isolated in situ as described previously [10,13].

2.2. Gel electrophoresis and hybridization analysis

Samples of agarose-embedded genomic DNA were digested with restriction enzymes at 37°C. Restriction endonucleases tested included *Age*I, *Bgl*I, *BssH*II, *Eag*I, *Mlu*I, *Msc*I, *NgoM*I, *Pac*I, *Pvu*II, *Sac*I, *Sal*I, *Xho*I, and *Xma*I (New England Biolabs, Beverly, MA, USA). Genomic DNA was separated by clamped homogeneous electric field gel electrophoresis and the DNA fragments stained with ethidium bromide, then visualized with UV illumination as described previously [13]. The sizes of restriction fragments were calculated by comparing their migration to those of two size standards: concatemers of λcI857 (monomer = 48.5 kb, New England Biolabs); and *Saccharomyces cereviseae* chromosomes (225–1900 kb, New England Biolabs). The DNA was fragmented in situ by UV-irradiation and depurinated before capillary transfer to nylon membranes (Hybond–N, Amersham Corp., Arlington Heights, IL, USA). Immobilized DNA was used to hybridize with radioactively labeled probes at 60°C and washed at 60°C with 2X SSC (1X SSC is 0.15 M NaCl, 0.015 M sodium citrate) plus 0.1% sodium doedecyl sulfate as described [10,13]. Hybridization probes were prepared from either recombinant plasmids or PCR products by random primer extension in the presence of [α-^{32}P] dATP (ca. > 3000 Ci/mmol, ICN Biomedical, Corp., Costa Mesa, CA, USA) using the Megaprime kit (Amersham Corp.). Gene targets used in this study are listed in Table 1.

2.3. Sequence analysis

Clones pSHH1, pSHH3, and pSHH6 span the *hlyA* gene from *B. hyodysenteriae* strain B204, and were described previously [11]. *B. pilosicoli* strain P43/6/78^T sequences from the *hlyA* locus were amplified by PCR using Taq polymerase (Boeringer Mannheim Corp. Indianapolis, IN, USA) and the products purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA, USA). Nucleotide sequences of plasmid and PCR templates were determined using dye termination sequencing reactions separated on Prism 377 DNA sequencers (Applied Biosystems, Foster City, CA, USA) at the Iowa State University Nucleic Acid Facility (Ames, IA, USA). Nucleotide sequence data was edited using Sequencher (Gene Codes Corp., Ann Arbor, MI, USA) and the genetic content analysed using Clone Manager (Scientific and Educational Software,

Table 1
Genes localized in *Brachyspira* genomes

Gene	Function	Species of origin	Probe reference
bit	Iron-regulated protein	*B. hyodysenteriae*	[18]
cheY	Chemotaxis	*B. hyodysenteriae*	AY573605[A]
clpX	Membrane protease	*B. pilosicoli*	[20], AY466377[A]
fabF	ACP synthase II	*B. hyodysenteriae*	U94886
fabF	ACP synthase II	*B. pilosicoli*	AY345132
fabG	ACP reductase	*B. hyodysenteriae*	U94886
fabG	ACP reductase	*B. pilosicoli*	AY345132
flaA	Flagella sheath protein	*B. hyodysenteriae*	[33]
*flaB*3	Flagellin core protein	*B. hyodysenteriae*	[34]
fliG	Motility switch protein	*B. hyodysenteriae*	AY573604[A]
fruBC	Phosphotransferase system enzyme IIBC	*B. hyodysenteriae*	[19]
gap	glyceraldehyde-3-phosphate dehydrogenase	*B. hyodysenteriae*	[34]
glt	Putative transport protein	*B. hyodysenteriae*	AY575208[A]
gyrA	DNA gyrase subunit A	*B. hyodysenteriae*	[10]
gyrB	DNA gyrase subunit B	*B. hyodysenteriae*	[35]
hlyA	Beta-hemolysin	*B. hyodysenteriae*	[11]
hlyA	ACP (homolog of *B. hyodysenteriae hlyA*)	*B. pilosicoli*	AY345132
mglB	Galactose/glucose binding protein	*B. pilosicoli*	[21]
nox	NADH Oxidase	*B. hyodysenteriae*	[36]
polA	DNA Polymerase I	*B. hyodysenteriae*	AF038544[A]
por	Pyruvate oxidoreductase	*B. pilosicoli*	[37]
ppiB	Cyclophylin	*B. hyodysenteriae*	AY575207[A]
rrf	5S rRNA	*Leptospira interrogans*	[27]
rrl	16S rRNA	*L. interrogans*	[27]
rrs	23S rRNA	*L. interrogans*	[27]
tlyA	Potential virulence factor	*B. hyodysenteriae*	[38]
tlyB	Clp-like protein	*B. hyodysenteriae*	[38]
tlyC	Putative hemolysin	*B. hyodysenteriae*	[38]
tpi	Triose-phosphate isomerase	*B. hyodysenteriae*	AP003190[B]
VSH-1	Gene transfer agent	*B. hyodysenteriae*	[12]
vspA	Variable surface protein A	*B. hyodysenteriae*	[16]
vspH	Variable surface protein H	*B. hyodysenteriae*	[17]

[A] Unpublished, GenBank accession numbers provided.
[B] Primers designed to amplify the *tpi* gene from *Clostridium perfringens* (AP003190) were used to amplify the *B. hyodysenteriae* ortholog.

Durham, NC, USA). Primers were designed using Primer Designer software (Scientific and Educational Software). *B. hyodysenteriae* and *B. pilosicoli hlyA* loci were compared using Clustal_X [14] and Genedoc (www.psc.edu/biomed/genedoc) software. Sequence homologies were determined using BLAST algorithms at http://www.ncbi.nlm.nih.gov/BLAST [15].

3. Results

3.1. Determination of the B. pilosicoli genome size

Restriction enzymes *Eag*I, *MluI*, *Sal*I, and *Xma*I each cut the *B. pilosicoli* genome into well-resolved fragments that were used to calculate its size (Fig. 1). *BssH*II recognized a single site in the *B. pilosicoli* genome that provided a unique landmark for subsequent mapping. Although *Age*I, *Bgl*I, *Msc*I, *NgoM*I, *Spl*I, and *Xho*I generated a few large fragments that were useful for constructing the physical map, these enzymes generated too many small fragments (<100 kb) to reliably calculate the size of the genome. The average calculated size of the *B. pilosicoli* genome is 2.45 Mb. In contrast, the *B. hyodysenteriae* genome was 3.2 Mb in size and thus contains about 750 kb more genetic material than *B. pilosicoli* [10].

3.2. Construction of a combined physical and genetic linkage map of B. pilosicoli

A restriction map of the *B. pilosicoli* genome was constructed with the enzymes *BssH*II, *Eag*I, *Sal*I, and *Xma*I (Fig. 2). Many of the *Mlu*I sites in the genome were also localized; however the positions of some *Mlu*I sites could not be determined because this enzyme also generates several small fragments. Two approaches were used to align restriction fragments generated with different enzymes. First, the products of double enzyme digests were compared to products generated with each enzyme alone. This approach allowed overlapping fragments to be identified (e.g. cf. lanes 3–5, Fig. 1), and was sufficient to localize the unique *BssH*II site. In addition, analysis of double digests showed that the

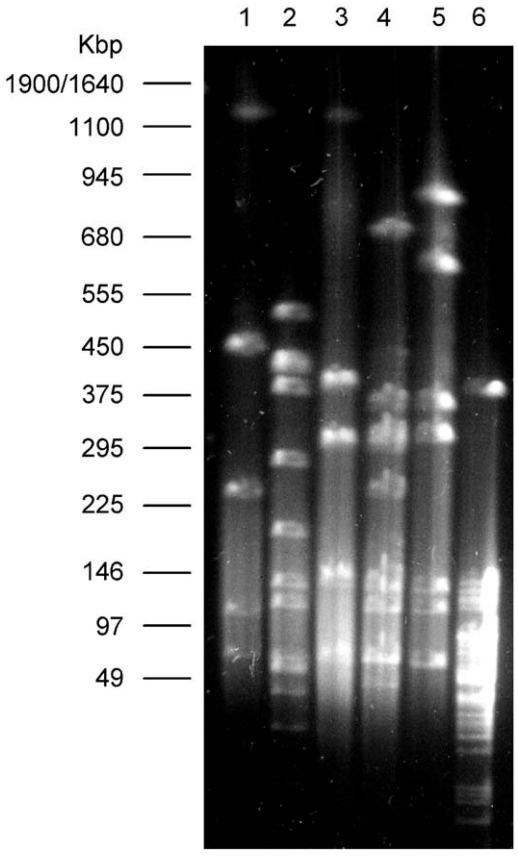

Fig. 1. Pulsed-field separation of *B. pilosicoli* restriction fragments. Genomic DNA was digested with *EagI* (lane 1), *MluI* (lane 2), *Sal*I (lane 3), *SalI* + *XmaI* (lane 4), *XmaI* (lane 5), and *NgoMI* (lane 6), separated in a 1% agarose gel, stained with ethidium bromide, and visualized by UV illumination. The migration of size standards is shown on the left.

largest fragments generated with *Eag*I, *Sal*I, and *Xma*I overlap. A second approach for mapping restriction sites used hybridization probes to known genes to confirm overlapping fragments, and resulted in simultaneous construction of physical and genetic maps of the genome (Fig. 2A). The resulting physical map was circular.

3.3. Comparison of Brachyspira genomes

The probes used to construct the *B. pilosicoli* map (Table 1) included a variety of housekeeping genes and several genes thought likely to affect host–parasite interactions. Localization of these genes also provided a more detailed map of the *B. hyodysenteriae* genome (Fig. 2B), thereby facilitating a comparison of genetic organization in these two species. These hybridization experiments were done under conditions of relaxed stringency that would accommodate about 20% sequence divergence. Copies of most genes tested were detected in both species. For example, all genes tested that related to motility and chemotaxis (e.g. *cheY*, *flaA*, *flaB*, and *fliG*) were detected in both species and are dispersed throughout each genome. In contrast, several genes detected in one species were not detected in the other. Specifically, the *B. hyodysenteriae* genes *vspA* and *vspH*, encoding variable surface proteins [16,17], *bitB*, part of an iron-regulated operon [18], and *fruBC*, a phosphotransferase adjacent to a putative fructose bisphosphate aldolase [19] were absent in *B. pilosicoli*. Conversely, two *B. pilosicoli* genes, *clpX* (a membrane

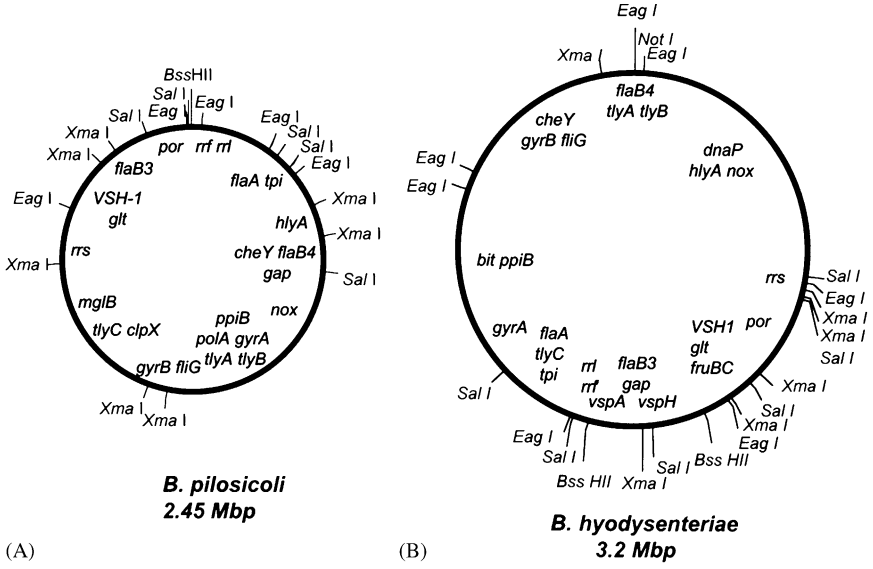

Fig. 2. Combined physical and genetic maps of *B. pilosicoli* strain P43/6/78^T (A) and *B. hyodysenteriae* strain B78^T (B). The physical maps are drawn to scale. The positions of restriction sites are shown outside the maps, with approximate locations for gene shown within the circular map boundary.

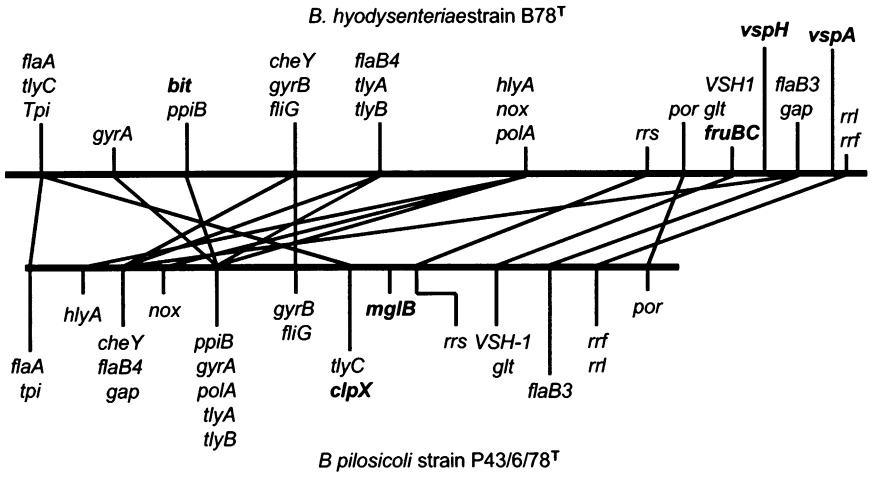

Fig. 3. Comparison of genetic organization of *B. pilosicoli* and *B. hyodysenteriae*. The two maps were rotated so that the *gyrA* and *gyrB* genes were in the same orientation, then the maps were linearized adjacent to the *flaA-tpi* locus. Genes are aligned between the two maps by lines. Genes present in one species, but not the other are shown in bold.

protease [20]) and *mglB* (a galactose glucose binding protein [21]) were not detected in *B. hyodysenteriae*.

To make a more detailed comparison, the maps of the *B. pilosicoli* and *B. hyodysenteriae* genomes were linearized adjacent to *flaA-tpi* and aligned at *gyrB* (Fig. 3). This orientation maximized the similarity between these two genetic maps. Next, lines were drawn between genetic loci common to both species to reveal differences in genetic organization. Several rearrangements are indicated by intersecting, diverging, or converging lines between the two maps (Fig. 3).

3.4. Comparative sequence analysis of hlyA loci

The production of strong zones of β-hemolysis on blood agar distinguishes *B. hyodysenteriae* from other intestinal spirochetes, including *B. pilosicoli.* The β-hemolysin was recently purified from *B. hyodysenteriae* and the gene encoding it, *hlyA*, was characterized [11]. The *hlyA* gene encodes an acyl-carrier protein (ACP), and although the mechanism by which the HlyA protein lyses erythrocytes is unknown, it is thought that β-hemolytic activity requires the mature, lipidated form of HlyA to lyse erythrocytes [11]. Because *B. pilosicoli* is not strongly hemolytic, and because *hlyA* sequences were not detected previously in *B. pilosicoli*, we were surprised to find *hlyA* sequences in the P43/6/78^T genome. The *hlyA* loci from *B. hyodysenteriae* and *B. pilosicoli* were sequenced to help identify genetic variations that may contribute to the differential phenotypes that distinguish these two species.

A 2.9 kb region encompassing the *B. hyodysenteriae* strain B204 *hlyA* gene was previously cloned [11], but only the *hlyA* gene sequence was determined. Sequence analysis of portions of plasmids pSHH1, pSHH3, and pSHH6 (described previously, 11), revealed two genes immediately adjacent to *hlyA*: *fabG* and *fabF*, encoding ACP-reductase and ACP-synthase II, respectively. *FabG*, *hlyA*, and *fabF* encode proteins that are well conserved among bacteria, and the genetic organization of this locus resembles similar loci from divergent bacterial genera [22]. Primers were used to amplify and sequence a 2464 bp region of the *B. pilosicoli* genome corresponding to the *B. hyodysenteriae fabG*, *hlyA*, and *fabF* genes. Attempts to amplify the 3′ end of the *fabF* gene were unsuccessful, suggesting that significant sequence divergence in the region downstream of *fabF* exists between *B. pilosicoli* and *B. hyodysenteriae.* Sequence analysis showed that the *hlyA* loci of *B. hyodysenteriae* and *B. pilosicoli* had the same organization, i.e., *fabG–hlyA–fabF* (Fig. 4A).

Alignment of the *hlyA*, *fabG*, and *fabF* genes revealed extensive regions of sequence identity (Fig. 4). The HlyA proteins from these two species were different at only two sites, and both were conservative amino acid substitutions (Fig. 4B). The ACP-reductase and ACP-synthase II of these two species showed more variation (Figs. 4C and D). There was substantial sequence variation between these two species in the intergenic regions surrounding *hlyA* that may also impact efficient transcription and translation of *hylA* and the downstream gene, *fabF*.

4. Discussion

This report presents the first detailed physical and genetic map of *B. pilosicoli* and the first comparison of genetic organization between two pathogenic species of *Brachyspira*. Through the construction of a combined physical and genetic map of the *B. pilosicoli* genome and improvement of the *B. hyodysenteriae* map, regions of

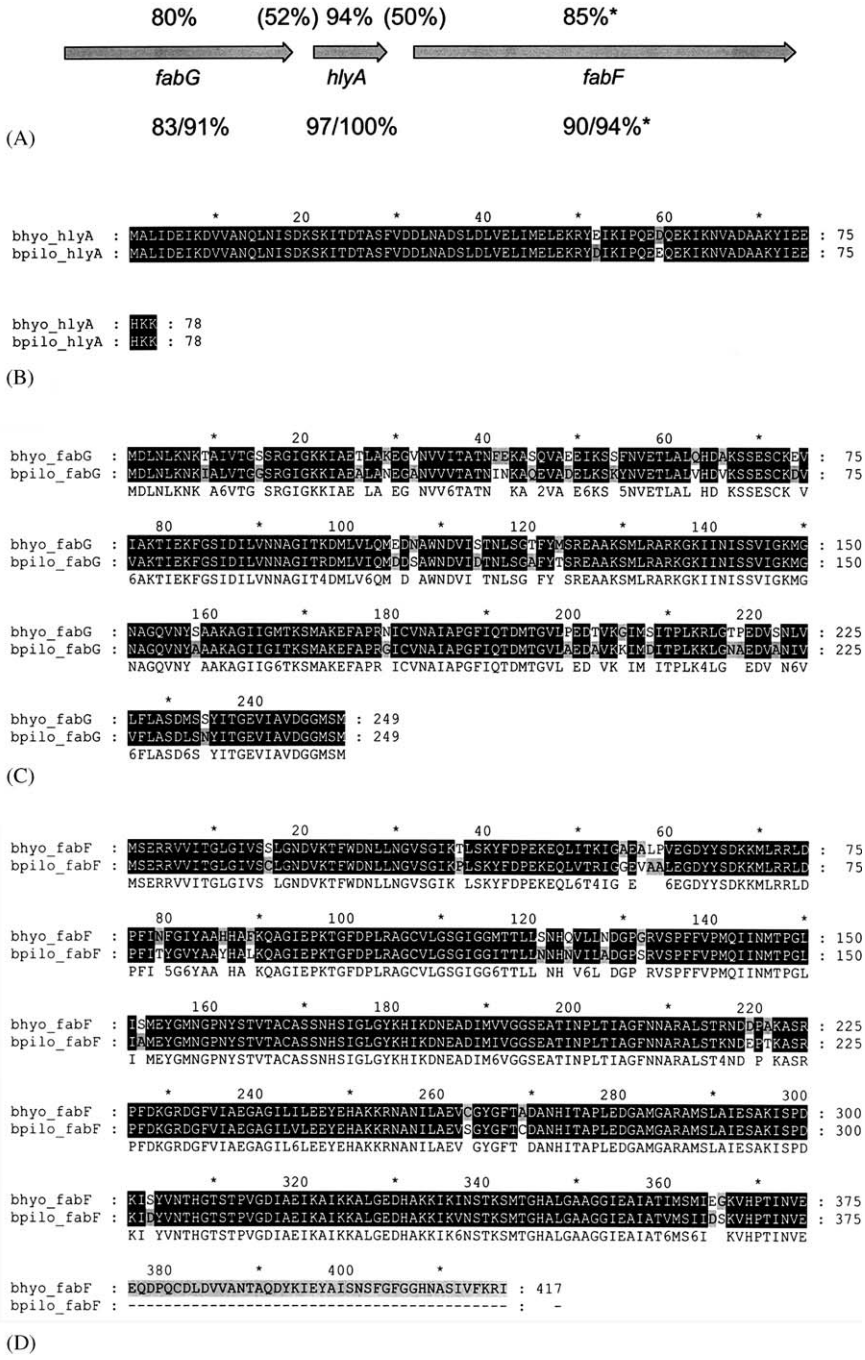

Fig. 4. Analysis of the *hlyA* locus. (A) A schematic of *Brachyspira fabG-hlyA-fabF* locus. Percentage nucleotide identity for each gene is shown above the schematic. Values in parentheses are intergenic regions. The percentage amino acid identity/similarity is shown below the schematic. (*The values given for *fabF* correspond only to the region sequenced in both species.) Amino acid sequences for HlyA (B), FabG (C), and FabF (D) were aligned using ClustalX, and the results drawn with GeneDoc. Consensus sequences shown under FabG and FabF include abbreviations for amino acid substitutions (2 = Q or E, 4 = R or K, 5 = F,Y, or W, and 6 = M,L,I, or V).

similarity and differences between these two species were identified. Several genes were present in one species but not the other. Furthermore, analysis of the *hlyA* loci of *B. hyodysenteriae* and *B. pilosicoli* highlighted differences and similarities between these bacteria that provide insight into phenotypic differences in these two species.

The *B. pilosicoli* genome was composed of a single circular chromosome, approximately 2.45 Mb in size. By comparison, the *B. hyodysenteriae* genome was approximately 750 kb larger than *B. pilosicoli*, enough DNA to contain over 700 genes of average size. Besides putative deletions or gene acquisitions that may account for these differences in size, the presence of intersecting lines

between the two genome maps in Fig. 3 shows evidence for rearrangements that differentiate these two species. The organization of the *rrl*, *rrs*, and *rrf* genes (encoding 23S, 16S, and 5S rRNAs, respectively) is common to both bacterial species. Each rRNA gene is present as a single copy, with *rrl* and *rrf* closely linked, yet separated from *rrs* by several hundred thousand bases (Fig. 1). This pattern of rRNA gene organization is unusual and distinguishes *Brachyspira* from other spirochetes [10].

The DNA gyrase gene is required for DNA replication. The subunits of bacterial gyrase are encoded by two genes, *gyrA* and *gyrB*, that are often located near the chromosomal origin of replication, *oriC*, and co-transcribed as a *gyrBA* transcript [23]. The *Brachyspira gyrA* and *gyrB* genes were separated by about 200–400 kbp (Fig. 2). Separation of these genes is not unique to *Brachyspira*: in the spirochete *Treponema pallidum*, these genes are separated by about 45 kbp [24]. In contrast, the *gyrA* and *gyrB* genes are adjacent in the spirochete species *Borrelia burgdorferi* [25] and *Leptospira interrogans* [26,27]. Further characterization of the regions surrounding the *Brachyspira* gyrase genes will be helpful to localize the chromosomal replication origin.

Several genes encoding proteins associated with metabolism and nutrient transport were localized. Most of the genes in these classes were found in both *B. hyodysenteriae* and *B. pilosicoli*, and localization of these genes allowed better resolution for comparing the two genomes. The location of *glt*, a putative transporter gene adjacent to VSH-1 sequences in *B. hyodysenteriae* (Stanton, T.B., Ames, 2002, unpublished data) was of particular interest because it provided a landmark to compare the location of VSH-1 insertion in these two species. The *glt* locus was also found near VSH-1 sequences in *B. pilosicoli*. These results indicate that insertion of VSH-1 in both species predates their divergence.

A key factor in *Brachyspira* colonization and virulence is the presence of functional flagella [28]. Both *B. pilosicoli* and *B. hyodysenteriae* are motile and both had all the motility and chemotaxis-related genes tested. However, these species differ in two carbohydrate utilization genes, *fru* and *mglB*, and both loci can influence chemotactic behavior and thereby affect growth and survival within selected niches of the intestinal environment. The fructose utilization (*fru*) locus was recently characterized in *B. hyodysenteriae*, but it is absent in *B. pilosicoli* [19]. Conversely, the *B. pilosicoli mglB* gene, encoding a galactose–glucose binding protein, is absent in *B. hyodysenteriae* [21]. *B. hyodysenteriae* is attracted towards porcine mucin [29], but the chemical composition of the attractant has not been elucidated. By identifying differences in the metabolic potential of these bacteria, it may be possible to develop an understanding of the specific requirements needed to direct these bacteria to a selected target in the intestine, and to establish infection.

The presence of β-hemolysis is a key element in distinguishing *B. hyodysenteriae* from other *Brachyspira*. The β-hemolysin responsible for this hemolytic activity is a small protein encoded for by the *hlyA* gene [11]. Initial hybridization analysis showed the presence of the *hlyA* gene in *B. pilosicoli*. However, because *B. pilosicoli* is weakly hemolytic [1,2], the discovery that this species has the β-hemolysin gene, *hlyA*, was unexpected (Fig. 2). A comparative analysis of the *hlyA* loci from *B. hyodysenteriae* and *B. pilosicoli* was done as a model to identify similarities and differences in potential virulence genes.

The HlyA proteins from *B. hyodysenteriae* and *B. pilosicoli* were found to be nearly identical, with only two conservative amino acid substitutions near the carboxy-terminus (Fig. 4B). These HlyA proteins also shared extensive sequence identity to ACPs of other bacteria (data not shown), that suggests they may have a similar structure. Because the *B. hyodysenteriae* and *B. pilosicoli* HlyA proteins were nearly identical, the question arises as to why *B. hyodysenteriae* is β-hemolytic whilst *B. pilosicoli* is only weakly hemolytic. One possibility is that differences in the chemical composition of lipid attached to the HlyA protein may affect its hemolytic activity (see below). Alternatively, sequence differences immediately upstream of the *hlyA* genes of these two species may alter transcription or translation of the *hlyA* gene in *B. pilosicoli*, resulting in reduced HlyA synthesis.

We propose that non-conservative sequence variation in *Brachyspira* FabF and FabG proteins (Figs. 4C and D) results in different lipid moieties being attached to HlyA proteins, thereby affecting hemolytic activity. ACP-reductase (FabG) and ACP-synthase II (FabF) play essential roles in the maturation of lipidated ACPs. Manipulation of *Pseudomonas aeruginosa* FabG activity using altered cofactors, or a temperature sensitive *fabG* mutant, resulted in the production of ACPs linked to a shorter fatty acid moiety [30]. Likewise, mutations in *fabF* altered the chemical composition of fatty acids attached to ACPs [31]. Variation in the amino acid sequences of *Brachyspira* ACP-reductase or ACP-synthase II may result in similar changes in the chemical composition or length of the lipid moiety attached to HlyA. Based on the structural analysis of the *E. coli* ACP [32], the lipid moiety is buried within four protein helicies that undergo a conformational change resulting in injection of the lipid into its target. While the mechanism of hemolysis caused by HlyA is unknown, attachment of an altered lipid moiety may affect its ability to destabilize the membrane when it interacts with an erythrocyte. Thus, variation in the chemical composition of lipid attached to HlyA may explain why

two bacteria with nearly identical HlyA protein sequences vary in their hemolytic activity.

Construction of the combined physical and genetic maps of *B. hyodysenteriae* and *B. pilosicoli* will aid efforts to sequence these bacterial genomes. Closure of genomic sequencing projects is the most labor intensive and time consuming component, and these maps provide a scaffold on which the genomic sequences can be assembled. Comparative analysis of these two species revealed differences that may have substantial influence on the ability of these bacteria to cause disease in specific hosts.

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